

symporter (vSGLT), a close structural homologue exhibiting the same topological fold. Based on the topological identity we also used the x-ray structure of Aquifex aeolicus leucine transporter (LeuT) to further improve the model of our system. Sequence alignment was performed to identify important residues and potential substrate/ion binding sites. Radioactive labeled biomarkers were used as potential substrates/inhibitors, in line with the clinical applications. Aside from the radio-labeled iodide, we considered tetrafluoroborate, perchlorate, bromide, and thiocyanate. Firstly, the binding sites were identified through a docking process. Following which in order to enhance I⁻ and inhibitor transport, necessary for improved imaging, we had to expand our system beyond the wild-type protein. The mutants we considered are all essential for transport and they are Q72N, I147C, E368A, and M68A. By inducing different rates of transport we can deduce how the protein responds to specific mutants. We have successfully identified a favorable response to our mutations and we are hopeful our approach to the engineered NIS protein will further aid in the treatment and imaging of thyroidal and extrathyroidal cancers.

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Effect of Mutations on Transport by the Sodium/Iodide Symporter (NIS)

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The Na⁺/I⁻ symporter (NIS) mediates iodide transport into the thyroid and other tissues, such as salivary glands, stomach, lactating breast, and small intestine. To understand the observed effects of single amino acid substitutions in the mechanism of transport by NIS, wild type NIS and NIS mutated at selected positions were studied using computational methods. Normal modes analysis with the Anisotropic Network Model (ANM) was used to explore the changes in global movements between NIS and the mutants in the presence or absence of the ions they transport (Na⁺, I⁻, and ClO₄⁻). ANM has been shown to be a useful computational tool for predicting the dynamics of membrane proteins in many applications. The lowest normal modes generated by the ANM provide valuable insight into the global dynamics of biomolecules that are relevant to their function. We employed a modified version of ANM in which closeness of side chains are also used to assign alpha-carbons connections, allowing us to identify differences due to mutations and the influence of ions on these global movements, thus enabling a first approximation to the influence of mutations on the transport mechanism of NIS.

To address questions relating to the coordination of the transported ions in the wild type and in mutants, we carried out molecular dynamics simulations in a lipid bilayer using an implicit solvent model with GBMV (Generalized Born Molecular Volume) in both wild-type NIS and NIS mutants and their ions. A homology model of NIS based on the structure of the bacterial homologue vSGLT (*Vibrio parahaemolyticus* Na⁺/galactose transporter) was used in both the normal modes analysis and the molecular dynamics simulations.

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Towards the Mechanism of Sodium/Proton Antiporter in *E. coli*

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NhaA is a secondary active transporter that regulates the cellular concentrations of proton and sodium ions. Sodium binding to two conserved aspartates Asp163 and Asp164 is thought to induce a conformational change from the inward- to the outward-facing state. The dimeric crystal structure of *E. coli* NhaA in the inward-facing state at acidic pH was recently determined. In contrast to the previous monomeric crystal structure, a salt bridge between the conserved residues Asp163 and Lys300 is formed, while the transmembrane helix that contains Lys300 is disrupted. To delineate the mechanism implicated by the structures, we calculate the pKa values of Asp163, Asp164 and Lys300 using all-atom continuous constant pH molecular dynamics simulations with pH-based replica-exchange sampling protocol. The results provide new insights into the working of the antiporter NhaA.

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The Molecular Basis for Substrate Specificity in Lactose Permease

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Lactose permease (LacY) has become a model system for monitoring substrate transport across the lipid bilayer. As a result of considerable experimental and structural data, the underlying molecular determinants for substrate specificity and affinity, as well as a mechanism for symport have been postulated. We have now monitored LacY structural dynamics in a lipid environment for 10 microseconds by using molecular dynamics simulations of a recent mutant LacY crystal structure trapped in a novel occluded state with bound high-affinity substrate. On this timescale, the sugar molecule exits the protein and re-enters. Therefore, the accompanying dynamics provide important clues regarding substrate specificity. In particular, Phe27 and neighboring lipid molecules assist in directing the sugar molecule to its binding position. In addition, simulations of substrates with different binding affinities enabled characterization of the structural framework governing substrate affinity in LacY.

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Defining the Conformational States in an Mfs Transporter, FucP

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The plethora of X-ray crystal structures in the Major Facilitator Superfamily has provided a series of snapshots that define the alternating access mechanism for ligand transport. A sticking point, however, is that there is not yet a series of conformational states obtained from one organism to support the proposed mechanism of transport. This is a problem that Molecular Dynamics (MD), using atomistic simulation, is ideally suited for to help improve the overall working hypotheses of how conformational changes in transmembrane helices occur during ligand transport.

FucP is the only X-ray crystal structure in an outward facing conformation and it is a L-fucose/H⁺ symporter, much like LacY. The resting state for this protein is thought to be accessible to the periplasm (outward facing) and the protonation of E135 in the central, solvent-accessible cavity along with the binding of the transported ligand, L-fucose is what destabilises the conformation, causing a movement to the inward-open state, allowing ligand transport into the cytoplasm.

Using MD simulation, the conformational changes FucP undergoes on ligand binding can be characterised and related to the specific roles of each of the 12 transmembrane helices. In particular helices 1 and 4 are believed to be pivotal to proton translocation via residues D46 and E135 [1], with E135 also involved in L-fucose binding. On protonation, the interaction between E135 and Y365 (helix 10) is destabilised and this is investigated using both biased and unbiased MD simulations to better define the degree to which the protein is 'open'.

1. Dang, S. et al., Structure of a fucose transporter in an outward-open conformation, (2010), *Nature*, 467, 734-738.

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The Human Proton-Coupled Folate Transporter: Determination of Conformation and Identification of the Folate-Binding Pocket

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Folate cofactors play crucial roles in hundreds of reactions in cells including DNA and protein synthesis. The human proton-coupled folate transporter (PCFT) is the only means of absorption of dietary folates in humans. PCFT expression and function is associated with many disorders including hereditary folate malabsorption, neural tube defects, Down syndrome, cancer, heart diseases, Alzheimer's and Parkinson's disease. Upregulation of PCFT expression in tumor cells is of significant consideration for development of PCFT-targeted chemotherapeutic agents. However, not much is known about the structure and function of PCFT, which contributes to the low clinical success rate of folate-based agents. To address this gap in the knowledge we performed extensive Cys-mutagenesis studies. We analyzed 40 residues towards the extracellular face of PCFT, 35 positions towards the cytoplasmic face of PCFT and 28 positions along the proposed folate-binding pocket of PCFT. Based on the accessibility studies of the extracellular face of PCFT we determined loop-helix boundaries of this face and identified the glycerol-3-phosphate transporter (PDB#1PW4) and tripeptide-proton symporter (PDB#4APS) as the best templates for modeling PCFT. Based on the accessibility studies of the cytoplasmic face of PCFT, we identified loop-helix boundaries of this face. Here, we also show that our accessibility studies support the hypothesis that PCFT is present predominantly in an inward-open conformation in the absence of substrate (pH 7.5 and no folic acid). We also show that the folate-binding pocket of PCFT is formed by residues present in PCFT transmembrane helices I, IV, V, X and XI. Our results are of high significance in understanding the details of folate-homeostasis mechanisms and in design of PCFT-targeted therapeutic and diagnostic agents.